Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

AYUSH- 64: A Potential Therapeutic Agent in COVID-19

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AYUSH- 64: A Potential Therapeutic Agent in COVID-19

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AYUSH- 64: A Potential Therapeutic Agent in COVID-19

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Keywords:	AYUSH-64; COVID-19; Corona Virus disease; Immunomodulation; SARS-CoV-2; Ayurveda
Abstract:	Corona Virus disease (COVID-19) becomes a global pandemic resulting in large scale morbidity and mortality worldwide. The causative agent SARS-CoV-2 is easily subject to repeated mutation with swift spread of infection. The management of COVID-19 was a big challenge on account of non-availability of specific therapeutic agents. The complex and multifactorial pathophysiology of COVID-19 requires therapeutic agents with anti-viral properties against SARS-CoV-2 as well as immunomodulatory properties that have a broad spectrum effectiveness covering the disease in totality. AYUSH-64, a polyherbal formulation developed by CCRAS, Ministry of AYUSH, Govt. of India through extensive pharmacological, toxicological and clinical studies has proven efficacy in infective febrile conditions such as malaria, microfilaremia, chikungunya and influenza with no safety issues observed in published clinical studies. Based on the empirical evidence, it has been repurposed as an adjuvant to standard care or standalone therapy for asymptomatic and mild to moderate cases of COVID-19 by the Ministry of AYUSH at a time when India is experiencing wave after wave of COVID-19 variants causing mass distress to the healthcare delivery systems. AYUSH-64 has four ingredients having immune-modulator, anti-inflammatory, antipyretic, antioxidant and anti-viral activities. These effects could arrest the extreme inflammatory responses in COVID-19 that causes progression to significant morbidity. Several clinical studies on AYUSH-64 in asymptomatic and mild to moderate cases of COVID-19 have been undertaken at reputed medical institutions across the country. The evidence generated through these studies is promising. AYUSH-64 has also been incorporated in the National COVID management protocol based on Ayurveda and Yoga by Government of India for asymptomatic and mild cases of COVID-19. Further, on the basis of tangible evidence generated through robust clinical and experimental studies on AYUSH-64, the Ministry of AYUSH has launched nation-wide campaign for mass distribution of AYUSH-64 to asymptomatic, mild to moderate COVID-19 patients in home isolation to reduce the burden on the hospital-based health care delivery system. This review will highlight about the specifications of AYUSH-64, its probable mechanism of action, its repurposing for COVID-19, various clinical and experimental studies undertaken during the COVID-19 pandemic and the initiatives taken to translate the outcomes of these studies on AYUSH-64.

AYUSH- 64: A Potential Therapeutic Agent in COVID-19

ABSTRACT

Corona Virus disease (COVID-19) has becomea global pandemic resulting in large scale morbidity and mortality worldwide. The causative agent SARS-CoV-2 is easily subjectto repeated mutation with swift spread of infection. The management of COVID-19 has been a big challenge on account of non-availability of specific therapeutic agents. The complex and multifactorial pathophysiology of COVID-19 requires therapeutic agents with anti-viral properties against SARS-CoV-2 as well as immunomodulatory properties that have a broad-spectrum effectiveness covering the disease in totality. AYUSH-64, a polyherbal formulation developed by CCRAS, Ministry of AYUSH, Govt.of India through extensive pharmacological, toxicological and clinical studies has proven efficacy in infective febrile conditions such as malaria, microfilaremia, chikungunya and influenza with no safety issues observed in published clinical studies.Based on the empirical evidence, it has been repurposed as an adjuvant to standard care or standalone therapy for asymptomatic and mild to moderate cases of COVID- 19 by the Ministry of AYUSH at a time when India is experiencing wave after wave of COVID-19 variants causing mass distress to the healthcare delivery systems. AYUSH- 64 has four ingredients having immune-modulator, anti-inflammatory, antipyretic, antioxidant and anti-viral activities. These effects could arrest the extreme inflammatory responses in COVID-19 that causes progression to significant morbidity. Several clinical studies on AYUSH-64 in asymptomatic and mild to moderate cases of COVID-19 have been undertaken at reputed medical institutions across the country. The evidence generated through these studies is promising. AYUSH-64 has also been incorporated in the National COVID management protocol based on Ayurveda and Yoga by Government of India for asymptomatic and mild cases of COVID-19. Further, on the basis of tangible evidence generated through robust clinical and experimental studies on AYUSH-64, the Ministry of Ayush has launched nation-wide campaign for mass distribution of *AYUSH-64* to asymptomatic, mild to moderate COVID-19 patients in home isolation to reduce the burden on the hospital-based health care delivery system. This review will highlight about the specifications of AYUSH-64, its probable mechanism of action, its repurposing for COVID-19, various clinical and experimental studies undertaken during the COVID-19 pandemic and the initiatives taken to translate the outcomes of these studies on AYUSH-64**.**

Keywords: AYUSH-64, COVID-19, Corona Virus disease, Immunomodulation, SARS-CoV-2, Ayurveda

1. Introduction

Historically, outbreak of infectious diseases offers some model of the course of diseases and treatment such as Spanish flu, Bird flu etc. which unfold over the time. Some diseases have predictable seasonal peak with high transmission as some pathogen may spread rapidly in dynamics of humidity, social mixing pattern leaving predictable pattern for each variant. The 'wave' of COVID-19 and its implications is the prominent topic of debate in current times. An epidemic wave/phase is defined as natural pattern of peaks and valleys which indicate the number of sick people and deaths in time frame and then decline.[1] The first wave peaked in India in September 2020 and second wave started from 1st week of March 2021. The second wave of COVID-19 tragedy and its impact on health care systems of our nation has been devastating and unprecedented.[2] There could be several factors responsible for the increased number of cases in the second wave. It is observed that the mutant strain of SARS-CoV-2 has higher transmission capability along with lesser incubation period. There has been a widespread disregard to the 'COVID Appropriate Behavior' by the general public, resulting in more severe illness, reduction in neutralizing antibodies and reduced effectiveness of vaccination.

The Ministry of AYUSH (MoA), Government of India has taken several public health and R&D initiatives to explore the potential of AYUSH systems to mitigate the impact of COVID-19 pandemic. Different institutions under the Ministry of AYUSH in collaboration with with prominent medical and research organizations across the country toundertake research on COVID-19 through various AYUSH systems. The MoA constituted an inter-disciplinary AYUSH R&D Task Force consisting of scientists, pulmonologists, epidemiologists, pharmacologists etc., from premier organizations and research institutions to handle various aspects of clinical and experimental research through AYUSH interventions. The Ministry of AYUSH also recommended a set of self-care guidelines for preventive health measures, with special emphasis on respiratory health and improving general immunity. The Ministry of AYUSH further issued the National Clinical Management Protocol based on Ayurveda & Yoga for management of COVID -19 to enable uniform clinical management.[3] The management guidelines advised in the protocol are based on the interim trends and outcomes of AYUSH COVID-19 studies along with the published evidence related to the safety and potential benefits of Ayush interventions.

2. Brief about AYUSH-64

AYUSH-64, a polyherbal formulation developed by Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH through extensive pharmacological,

toxicological and clinical studies. It has proven efficacy in infective febrile conditions such as malaria, microfilaremia, chikungunya and influenza with no safety issues observed in published clinical studies.[4-9] Further, AYUSH 64 was found safe and non-toxic in a dose of 500 mg/kg body weight for 12 weeks in experimental studies.[10-11] Quality control and safety parameters of the ingredients of AYUSH-64 and the final formulation have been complied with the Ayurveda Pharmacopoeia limits. The constituents of AYUSH-64 viz. *Saptaparna* (*Alstonia scholaris* R. Br.), *Katuki* (*Picrorhiza kurroa* Royle ex. Benth), *Kiratatikta* (*Swertia chirata* Pexbex. Karst) and *Kuberaksha* (*Caesalpinia crista* L.) are reported to possess immunomodulatory, anti-inflammatory, antipyretic, antioxidant and anti-viral activities.[12-17] These effects could inhibit the extreme inflammatory responses in COVID-19 that causes progression to significant morbidity. In a prospective, open-label, non-randomized pre-test and post-test design pilot study on AYUSH-64 in Influenza like Illness (ILI), one-week intervention of AYUSH-64 in a dose of 3 gm/day provided early clinical recovery from Influenza Like Illness (ILI) symptoms with reduced frequency of usage of acetaminophen/ antihistaminic.[9] The results of a Molecular Docking study revealed that the presence of M^{pro}-Akuammicine N-Oxide with highest M^{pro} binding energy along with other 34 phyto-constituents having similar anti-viral activity against SARS- CoV-2as part of AYUSH-64 make it a suitable drug/medicine for repurposing for the COVID-19 management.[18]

New indication for COVID-19

AYUSH-64 was repurposed for COVID-19 based on the recommendations of Interdisciplinary AYUSH R & D Task Force constituted by the Ministry of AYUSH along with available quality standards, evidences on clinical and pre-clinical safety[4-8,10-11], evidences on efficacy in Influenza-like illness[9] . The new indication was based on anti-viral and immunomodulator activity[12-17] as well as evidences drawn through above mentioned molecular docking study[18]. As per the clinical trial registry of India, total seven clinical studies on AYUSH-64 in asymptomatic and mild to moderate COVID-19 cases were undertaken by reputed medical institutions across the country. AYUSH-64 was administered in the management of asymptomatic & mild cases of COVID-19 as standalone treatment and for the management of mild and moderate COVID-19 as an adjunct to standard care in these clinical trials. The outcomes of these studies demonstrated that AYUSH-64 as adjunct treatment to standard care resulted in early clinical recovery compared to standard of care alone without progression of the disease to severe or critical stage.[19-22] The mean time to negative RT-PCR assay for COVID-19 was also better in the AYUSH-64 add-on group.[19,21]. Also, there was improvement in Quality of life (QoL) parameters.[19] AYUSH-64 was found to be well tolerated and safe. Based on the leads generated through experimental and clinical studies, AYUSH-64 has been incorporated in the National COVID management protocol based on Ayurveda and Yoga for asymptomatic and mild cases of COVID-

19.[3] AYUSH-64 is further included in the "Guidelines for Ayurveda Practitioners for COVID-19 Patients in Home Isolation" issued by the Ministry of AYUSH during the second outbreak of COVID-19 in the country.[23] The Ministry of AYUSH has also launched nation-wide campaign for mass distribution of *AYUSH-64* to asymptomatic, mild to moderate COVID-19 patients in home isolation to reduce the burden on the hospital-based health care delivery system.[24]

The objective of this review is to scientifically explain the general therapeutic approach of AYUSH-64 to intervene in disease progression during various clinical and pathogenic stages of COVID-19.

3. COVID-19: Window of Transmission and Infection Dynamics

SARS-CoV-2 virus spreads through respiratory droplets of infected individuals. The average incubation period ranges from 1 to 14 days and mean is (5 to 6 days).[25] The chances of spread of infection are higher during the initial period of infection; however patient can remain infected for an average of two weeks. The window of infected state varies from person to person; some individual may remain infected for several weeks and remain asymptomatic known as long term spreader. A large proportion of individuals affected with COVID-19 are infected either with pre-symptomatic or with asymptomatic transmission. So, keeping track of these variants is very vital for this pandemic control. Nearly 80% persons have been asymptomatic or display mild symptoms, 15% display moderate to severe symptoms and require oxygen support and 5% patients have been critical with an immediate need for mechanical ventilation even during second outbreak of COVID-19. The mortality varies from country to country and is approximately 11% worldwide in hospitalized patients, although it was less than 1.1%in India.[26]

The viral dynamics reveal that there is no difference of viral load and severity of COVID-19 and its clinical outcome. The infection clears by itself in early stage in mild cases, while severe cases have prolonged viral shedding. The studies so far have shown conflicting evidence in regard to the viral shedding kinetics. The patient can continue to shed the virus even after the symptom resolution. The mean duration of viral shedding is 20 days among the survivors. It is also noted that some patients with mild symptoms of COVID-19 initially can suffer from variable and debilitating symptoms for more than two months of the initial infection referred as long-term effects of COVID-19.[27]

3.1 COVID-19: Clinical features and immune response

The clinical features vary from patients to patients depending on the individual's immune response**.** The clinical category of COVID-19 include asymptomatic (positive RT-PCR without symptoms), mild to moderate (positive with clinical manifestations) or severe and critical (positive with high degree of manifestations)**.**The common

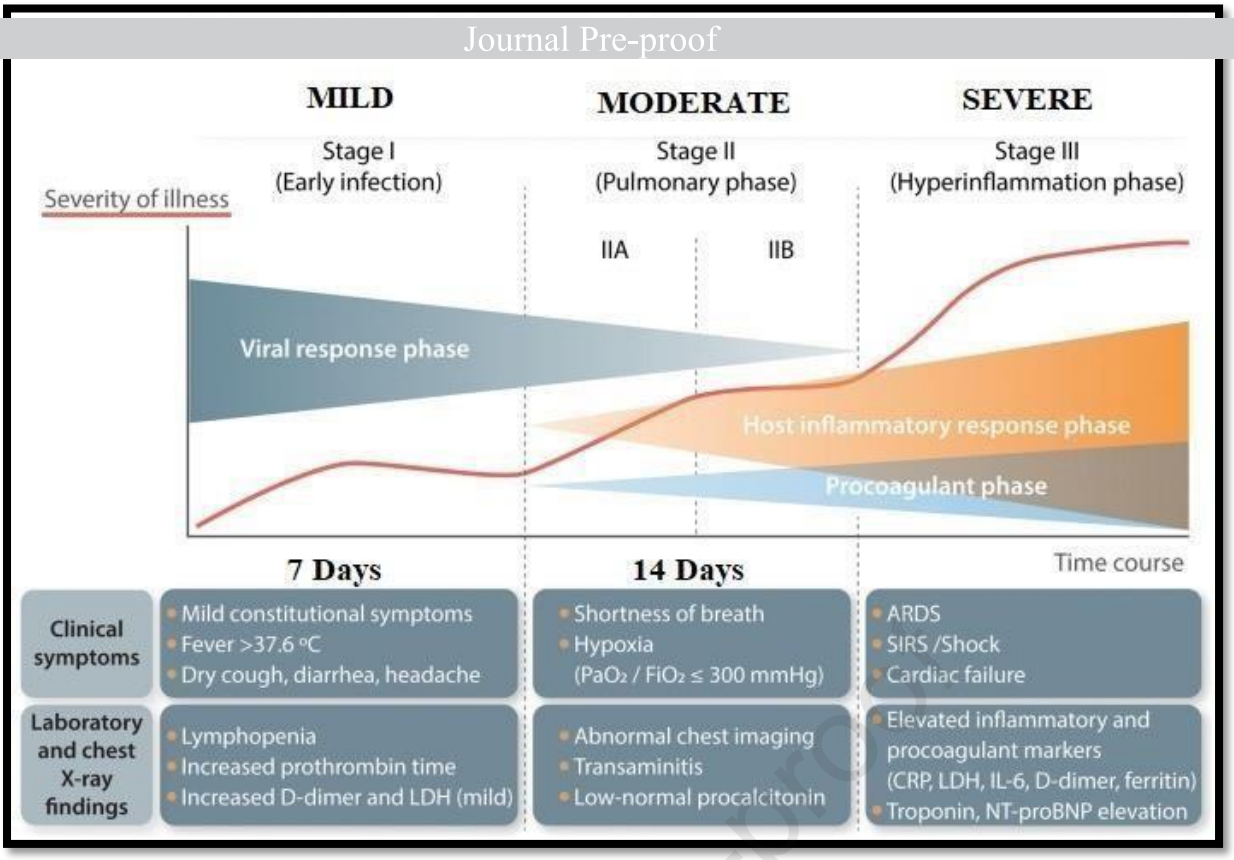
symptoms of COVID-19 include fever (83-98%), fatigue (70%), dry cough (82%), headache (34%), dyspnea (50%), sore throat (14%), rhinorrhea (7%), anosmia (Loss of smell) and ageusia (Loss of taste) (< 7%), diarrhea (20%), vomiting (14%) and weakness (70%) in the hospitalized patients[28-30] along with a significant increase of C-reactive protein (>20mg/dl). The CRP concentration is more in severe (>40mg/dl) and critical cases (>100mg/dl) compared to mild cases (< 18mg/dl).[31] The raised absolute lymphocyte count (<100cells/µl) is also found in severe patients.[32] The increased respiratory rate >30/min and SpO2 <90% are the indicators of severe COVID-19. Some patients proceed to critical stage as observed by systemic inflammation due to cytokine storm and widespread platelet aggregation leading to multi-organ failure as the end stage of COVID-19.

An infected person initially moves through stages of replication over first few days followed by a stage of adaptive immunity over the next few days. In the replication stage, the virus replicates leading to flu-like illness characterized by mild symptoms due to direct cytopathic effect (structural changes in host cells) of the virus. In the stage of adaptive immunity, virus levels decline as immune system takes over, but in inflammatory phase, there is possibility of cytokine storm leading to tissue destruction and clinical deterioration.[33]

3.2. Pathological progression of COVID-19

To develop effective therapeutics and preventive measures against COVID-19, an accurate and precise understanding of its pathogenesis at the molecular level is needed. Based on the most recently published literature, the overall COVID-19 pathogenesis process can be summarized as three clinically distinct and potentially overlapping phases. As shown in Figure 1, its pathological progression can be classified in chronological order as viral replication or early infection, pulmonary, proinflammatory, and prothrombotic phases.[34]

Figure 1: Clinical stages of COVID-19



4. Probable mechanism of action of AYUSH-64

The chemical constituents and therapeutic indications of the four ingredients of AYUSH-64 are given in Table 1. As per the published evidence, the ingredients of AYUSH-64 have the potential to control the initial symptoms of COVID-19, control the cytokine storm through the inhibition of cytokines, inhibition of Angiotensin-Converting Enzyme II (ACE2) and Reactive Oxygen Species (ROS).

4.1 Anti viral activities Isolated molecules of AYUSH-64

The isolated molecules of medicinal plants present in AYUSH-64 have antiviral activities against COVID-19 by interfere with the important amino acids to inhibit the activity of spike glycoprotein of SARS-CoV-2 as well as ACE2. The α-amyrin and Alstonides present in saptoparna , amarogentin, eufoliatorin, , kutkin present in Katiki and caesalpinins in Kuberakshya (Table-1) are the top ranked molecules have the highest affinity towards both the spike glycoprotein and ACE2[35].

Table 1:Chemical constituents and indications of four constituents in AYUSH-64

Name of the Medicinal Plant	Major Chemical Constituents	Indication as per Ayurveda
Saptaparna (*Alstonia scholaris* R. Br.)	Akuammicine N-Oxide, Akuammiginone, Echitaminic acid, Echitamidine N-oxide, Alstonides, alpha -amyrin	*Jvara (disease conditions associated with pyrexia), Kasa (cough), Swasa (dyspnea), Pravahika (dysentry)*
Katuki (*Picrorhiza kurroa* Royle ex. Benth)	Picroside I and II, kutkoside, cucurbitacins, vanillic acid, cinnamic acid,ferulic acid, apocynin , saptoparna , amarogentin, eufoliatorin, , kutkin	*Visamajvara (disease conditions associated with recurrent pyrexia), Kasa (cough), Swasa (dyspnea)*
Kiratatikta (*Swertia chirata* Pexbex. Karst)	Xanthine, Xanthine glycoside, flavonoids, iridoid glycocide, triterpenoid	*Visamajvara (disease conditions associated with recurrent pyrexia), Kasa (cough), Swasa (dyspnea)*

Kuberaksha (*Caesalpinia crista* L.)	Diterpines, caesalpinin N, Diterpenoids, Casalmin,	*Jvara (disease conditions associated* (*cough), Swasa (dyspnea), Sopha (inflammation)*

4.2 Effect of AYUSH-64 in SARS-CoV-2 induced pro-inflammatory process

The majority of COVID-19 patients have subnormal or reduced leucocyte count, lymphocytopenia, increased interleukin and tumour necrosis factor α. SARS-CoV-2 virus enters through ACE2 via TLR-7 (Tell like Receptor-7) which activates the pro-inflammatory kinases. AYUSH -64 has been rich in phytoacids with acidic pH. The anti-viral activities of AYUSH-64 may be achieved by increased pH of intra-cellular vacuoles and decreased endosomal activities like other anti-malarial drugs.[36] The immediate immune responses to COVID-19 are deregulation of central metabolism for mobilization of energy, cells and biomolecules.[37-39] This deregulation of central metabolism is called *Amadosha* (undigested byproducts of digestion and metabolism) in Ayurveda. *Saptaparna* and *Katuki* have *Deepana* and *Pachana* properties (enhancing digestion and metabolism) which clear the *Amadosha*. So, AYUSH-64 maybe reprogramming the host metabolism and regulate the enzymatic activities and biosynthesis to generate antiviral defense response. Moreover, anti-inflammatory and antioxidant activities of *Swertia chirata Pexbex. Karst* potentiates its action.[40]

4.3 Effect of AYUSH-64 on potential agents for pulmonary impairment

COVID-19 begins in upper respiratory tract but replicates very fast in ACE2 receptor rich respiratory mucosa. After touching the alveoli, virus begins to replicate fast in pneumocyte causing cell death. This cellular damage accelerates the appearance of multinucleated giant cells and fibrin rich hyaline membrane. All the phytoconstituents of AYUSH-64 have cough and dyspnea relieving properties which reverse the inflammatory process and reduce fibrosis. The concept of *Pramathi* (clearing property of cell debrides) is extremely useful for downward regulation of fibrin deposition. The alkaloids from *Alstonia scholaris* R. Br. inhibit Influenza A virus replication and lung immunopathology by regulating the innate immune response.[41-42] Research studies found that Neutrophil Extracellular Traps (NET) is the main culprit of pulmonary dysfunction and AYUSH-64 decreases the total neutrophil counts of COVID-19 patients. The clinical indicators of COVID-19 patients (fever, cough and dyspnea) decreased in AYUSH-64 group proving that AYUSH-64 has a promising efficacy in preventing the lung impairment.[43-46] *Caesalpinia crista* L. has excellent therapeutic potential to reduce fever, cough and clears the lungs.[47]

4.4 Effect of AYUSH-64 in SARS-CoV-2 induced hyperinflammatory and prothrombic phase

The inflammatory phase of COVID-19 passed through various mediators activates haemocytic system through endothelial dysfunction, platelet activation and micro and macro vascular thrombosis. Human platelets express through ACE2 and TMPRSS2 receptors. SARS-CoV-2 and its Spike protein directly enhance the platelet activation by binding to these receptors.[48] *Picrorhiza kurroa* Royle ex. Benth in AYUSH-64 possess thrombolytic action as described in Ayurveda. Another view is that there is an elevation of D-dimer in critical patients of COVID-19 and AYUSH-64 decrease the levels of D-dimer significantly. Immunosuppressive effect *of Swertia chirata Pexbex. Karst* may reduce the inflammatory process.[49]

5. CONCLUSION

The ingredients of AYUSH-64 have the potential to control the initial symptoms of COVID-19 and the cytokine storm, inhibition of the Angiotensin-Converting Enzyme II (ACE2) and Reactive Oxygen Species (ROS) and thereby prevention of the progression of COVID-19. Taking leads from the outcomes of clinical study on influenza-like illness and molecular docking study, AYUSH-64 has been repurposed for the management of asymptomatic and mild to moderate cases of COVID-19 as standalone therapy or adjunct to standard care in several clinical trials with promising results. Thus, AYUSH-64 could play a significant role in reducing the large-scale morbidity associated with COVID-19 and reduce the burden on the hospital-based health care delivery system by effectively managing the home-isolated cases.

REFERENCES

1. Dhar Chowdhury S, Oommen AM. Epidemiology of COVID-19. *Journal of Digestive Endoscopy*. 2020;11(1):3-7. doi:10.1055/s-0040-1712187

2. Jain VK, Iyengar KP, Vaishya R. Differences between First wave and Second wave of COVID-19 in India [published online ahead of print, 2021 May 8]. *Diabetes MetabSyndr*. 2021;S1871-4021(21)00147-8. doi:10.1016/j.dsx.2021.05.009

3. Ministry of AYUSH, Government of India. National Clinical Management Protocol based on Ayurveda and Yoga for management of Covid-19. Available from: https://www.ayush.gov.in/docs/ayush-Protocol-covid-19.pdf; 2020.

4. Chari MV, Venkataraghavan S, Seshadri C, Shetty BR, Gowri N. A double-blind clinical trial with Ayush-64 an Ayurvedic drug in P. vivax Malaria. Jour. Res. Ay. Sid. 1982; 6 (3-4): 105-116

5. Bhatia D. Role of AYUSH-64 in malaria epidemic. Jour. Res. Ay. Sid. 1997; 18 (1-2): 71-76.

6. Sharma KD, Kapoor ML, Vaidya SP, Sharma LK. A Clinical Trial of “Ayush-64” (A Coded Antimalarial Medicine) in Cases of Malaria. Jour. Res. Ay. Sid., Vol. II. No. 4

7. Pandey PN, Kishore P. Effect of Ayush-64 and SaptaparnaghanaVati on Microfilaraemia. Jour. Res. Ay. Sid. 1989; 12(3-4): 145-150.

8. Anonymous. Management of chikungunya through Ayurveda and Siddha- A technical report. Central Council for Research in Ayurvedic Sciences, Department of Ayush, Ministry of Health & Family Welfare, Government of India, 2009.

9. Gundeti MS, Bhurke LW, Mundada PS, Murudkar S, Surve A, Sharma R, et al. AYUSH 64, a polyherbal Ayurvedic formulation in influenza like illness: results of a pilot study. J Ayurveda Integr Med 2020. https://doi.org/10.1016/j.jaim.2020.05.010.

10. Anonymous. Ayush-64 A New Ayurvedic Anti- Malarial Compound. Central Council for Research in Ayurveda and Siddha, Ministry of Health & Family Welfare, Govt. of India 1987.

11. Anonymous. Pharmacological Investigations of certain Medicinal Plants and Compound Formulations used in Ayurveda and Siddha. Central Council for Research in Ayurveda and Siddha, Ministry of Health & Family Welfare, Govt. of India 1996.

12. Zhao YL, Shang JH, Pu SB, Wang HS, Wang B, Liu L, et al. Effect of total alkaloids from Alstonia scholaris on airway inflammation in rats. J Ethnopharmacol. 2016; 178:258-65. doi: 10.1016/j.jep.2015.12.022.

13. Hu TY, Ju JM, Mo LH, Ma L, Hu WH, You RR, et al. Anti-inflammation action of xanthones from Swertia chirayita by regulating COX-2/NF-κB/MAPKs/Akt signaling pathways in RAW 264.7 macrophage cells. Phytomedicine. 2019;55:214-221. doi: 10.1016/j.phymed.2018.08.001.

14. Shukla S, Mehta A, Mehta P,Vyas SP, Shivaprasad HN. In vivo

immunomodulatory activities of the aqueous extract of bonduc nut Caesalpinia bonducella seeds. Pharm Biol. 2010;48(2):227-30. doi:

15. Shukla S, Mehta A, Mehta P, Vyas SP, Shukla S, Bajpai VK. Studies on anti-inflammatory, antipyretic and analgesic properties of Caesalpinia bonducella F. seed oil in experimental animal models. Food Chem Toxicol. 2010;48(1):61-64. doi: 10.1016/j.fct.2009.09.015.

16. Gupta A, Khajuria A, Singh J, Bedi KL, Satti NK, Dutt P, et al. Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from Picrorhiza kurroa. Int Immunopharmacol. 2006;6(10):1543-9. doi: 10.1016/j.intimp.2006.05.002.

17. Kumar R, Gupta YK, Singh S, Raj A. Anti-inflammatory Effect of Picrorhiza kurroa in Experimental Models of Inflammation. Planta Med. 2016;82(16):1403-1409. doi: 10.1055/s-0042-106304.

18. Ram TS, Munikumar M, Raju VN, Devaraj P, Boiroju NK, Hemalatha R, et al. In silico evaluation of the compounds of the ayurvedic drug, AYUSH-64, for theaction against the SARS-CoV-2 main protease. J Ayurveda Integr Med 2021. https://doi.org/10.1016/j.jaim.2021.02.004.

19. Chopra A, Chavan-Gautam P, Tillu G, Saluja M, Borse S, Sarmukaddam S, et al. Coadministration of AYUSH 64 as an adjunct to Standard of Care in mild and moderate COVID-19: A randomised, controlled, multicentric clinical trial. Preprint available at: https://www.medrxiv.org/content/10.1101/2021.06.12.21258345v1

20. Reddy RG, Gosavi RV, Yadav B, Rai AK, Holay MP, Talekar M et al. AYUSH- 64 as an add-on to standard care in asymptomatic and mild cases of COVID- 19: A randomized controlled trial. Preprint available at: https://osf.io/pgraf/

21. Singh H, Srivastava S, Yadav B, Rai AK, Jameela S, Muralidharan S et al. AYUSH-64 as an adjunct to Standard Care in mild to moderate COVID-19: An open-label randomized controlled trial in Chandigarh, India. Preprint available at: https://osf.io/xyuas/

22. Bhardwaj P, Godatwar PK, Charan J, Sharma S, Shafi S, Chauhan N et al. Efficacy and Safety of Ayurveda Intervention (AYUSH 64) as add-on therapy for patients with COVID-19 infections – An open labelled, Parallel Group, Randomized controlled clinical trial. Preprint available at: https://www.medrxiv.org/content/10.1101/2021.08.10.21261836v1

23. Ministry of AYUSH, Government of India. Ayurveda Preventive Measures for self-care during COVID-19 Pandemic. Available from: https://www.ayush.gov.in/docs/Ayurveda%20Preventive%20Measures%20for%20self%20care%20during%20%20COVID-19%20Pandemic.pdf; 2021

24. Press Information Bureau, Government of India. Ayush Ministry launches nationwide distribution campaign of AYUSH 64 & Kabasura Kudineer. 7th May 2021. Available from: https://pib.gov.in/Pressreleaseshare.aspx?PRID=1716729

25. Machhi J, Herskovitz J, Senan AM, et al. The Natural History, Pathobiology, and Clinical Manifestations of SARS-CoV-2 Infections. *J Neuroimmune Pharmacol*. 2020;15(3):359-386. doi:10.1007/s11481-020-09944-5

26. Carbone M, Lednicky J, Xiao SY, Venditti M, Bucci E. Coronavirus 2019 Infectious Disease Epidemic: Where We Are, What Can Be Done and Hope For. *J Thorac*

27. Zacharioudakis IM, Prasad PJ, Zervou FN, et al. Association of SARS-CoV-2 Genomic Load with Outcomes in Patients with COVID-19. *Ann Am Thorac Soc*. 2021;18(5):900-903. doi:10.1513/AnnalsATS.202008-931RL

28. Jiang F, et al. Review of the clinical characteristics of coronavirus disease 2019 (COVID-19) *J. Gen. Intern. Med.* 2020;**35**:1545–1549. doi: 10.1007/s11606- 020-05762

29. Sharma R, Agarwal M, Gupta M, Somendra S, Saxena SK. Clinical Characteristics and Differential Clinical Diagnosis of Novel Coronavirus Disease 2019 (COVID- 19). *Coronavirus Disease 2019 (COVID-19)*. 2020;55- 70. Published 2020 Apr 30. doi:10.1007/978-981-15-4814-7_6

30. Romagnoli S, Peris A, De Gaudio AR, Geppetti P. SARS-CoV-2 and COVID- 19: From the Bench to the Bedside. *Physiol Rev*. 2020;100(4):1455-1466. doi:10.1152/physrev.00020.202

31. Ali N. Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. *J Med Virol*. 2020;92(11):2409-2411. doi:10.1002/jmv.26097

32. Wagner J, DuPont A, Larson S, Cash B, Farooq A. Absolute lymphocyte countis a prognostic marker in Covid-19: A retrospective cohort review. *Int J Lab Hematol*. 2020;42(6):761-765. doi:10.1111/ijlh.13288

33. Oliveira DS, Medeiros NI, Gomes JAS. Immune response in COVID-19: What do we currently know?. *MicrobPathog*. 2020; 148:104484. doi:10.1016/j.micpath.2020.104484

34. Yu C, Zhou M, Liu Y, et al. Characteristics of asymptomatic COVID-19 infectionand progression: A multicenter, retrospective study. *Virulence*. 2020;11(1):1006-1014. doi:10.1080/21505594.2020.1802194

35. Maurya VK, Kumar S, Bhatt MLB, Saxena SK. Antiviral activity of traditional medicinal plants from Ayurveda against SARS-CoV-2 infection [published online ahead of print, 2020 Oct 19]. *J Biomol Struct Dyn*. 2020;1-17. doi:10.1080/07391102.2020.1832577

36. Thakar A, Panara K, Patel F, Bhagiya S, Goyal M, Bhinde S, Chaudhari S, Chaturvedi S. Add-on Ayurveda Treatment for Early Stage COVID-19: A Single Center Retrospective Cohort Study From Gujarat, India. J Evid Based Integr Med. 2021;26:2515690X211020685. doi: 10.1177/2515690X211020685.

37. Cheng S.-C., Joosten L.A.B., Netea M.G. The interplay between central metabolism and innate immune responses. *Cytokine Growth Factor Rev.* 2014;25:707–713. doi: 10.1016/j.cytogfr.2014.06.008.

38. Hashimoto T, Perlot T, Rehman A, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature. 2012;487:477–81

39. Singh N, Suthar B, Mehta A, Nema N, Pandey A (2020) Corona Virus: An doi.org/10.23937/2378-3672/1410050

40. Das, S. C., Bhadra, S., Roy, S., Saha, S. K., Islam, M. S., and Bachar, S. C. (2012). Analgesic and anti-inflammatory activities of ethanoic root extract of *Swertia chirata* (Gentianaceae). *Jordan J. Biol. Sci.* 5, 31-36.

41 Zhou HX, Li RF, Wang YF, Shen LH, Cai LH, Weng YC, Zhang HR, Chen XX, WuX, Chen RF, Jiang HM, Wang C, Yang M, Lu J, Luo XD, Jiang Z, Yang ZF. Total alkaloids from Alstonia scholaris inhibit influenza a virus replication and lung immunopathology by regulating the innate immune response. Phytomedicine. 2020;77:153272. doi: 10.1016/j.phymed.2020.153272.

42 Nguyen PQ, Ooi JS, Nguyen NT, et al. Antiviral Cystine Knot α-Amylase Inhibitors from Alstoniascholaris. J Biol Chem. 2015;290(52):31138- 31150. doi:10.1074/jbc.M115.654855

43 Najmi A, Javed D, Ray A, Sadasivam A. Review of Antiviral Activities Present in some Indian Medicinal Plants – Can they be used against SARS-CoV-2?. Int Arch BioMed Clin Res. 2020;6(4):RA1-RA5

44 Turaskar A, More S, Sheikh R, Gadhpayle J, Bhongade SL. Inhibitory potential of *Picrorhizakurroa* royle ex. benth extracts on phenylhydrazine induced reticulocytosis in rats. Asian J of Pharm Clin Res 2013;6(2):215-16.

45 Zhang DK, Yu JJ, Li YM, et al. A Picrorhiza kurroa derivative, picroliv, attenuates the development of dextran-sulfate-sodium-induced colitis in mice. *Mediators Inflamm*. 2012;2012:751629. doi:10.1155/2012/751629

46 Kumar V, Singh SB, Singh S. Pharmacological Perspectives of Ayurvedic herbs viz. Alstonia scholaris L., Picrorhiza kurroa, Swertia chirata and Caesalpinia crista against COVID- 19: A Mini-Review. Mini-Reviews in Organic Chemistry 2021;18:1-9.
https://doi.org/10.2174/1570193X17999201102200944

47 Eric Wei Chiang entitled *Caesalpinia Crista:* A costal woody Climber with promising therapeutic values, Journal of Applied Pharmaceutical Science 8(3):133-140 10.7324/JAPS.2018.8319

48 Zhang S, Liu Y, Wang X, Yang L, Li H, Wang Y, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol. 2020 Sep 4;13(1):120. doi: 10.1186/s13045-020-00954-7.

49 Ahirwal L, Singh S, Bharti V and Mehta A: Immunosuppressive effect *ofSwertia chirata* Buch Ham. on Swiss albino mice. *Int J Pharm Sci Res* 2013; 4(12): 4763-68. doi: 10.13040/IJPSR. 0975-8232.4(12).4763-68

1. Anonymous. Ayush-64 A New Ayurvedic Anti- Malarial Compound. Central Council for Research in Ayurveda and Siddha, Ministry of Health & Family Welfare, Govt. of India 1987.

2. Anonymous. Pharmacological Investigations of certain Medicinal Plants and Compound Formulations used in Ayurveda and Siddha. Central Council for Research in Ayurveda and Siddha, Ministry of Health & Family Welfare, Govt. of India 1996.

3. Zhao YL, Shang JH, Pu SB, Wang HS, Wang B, Liu L, et al. Effect of total alkaloids from Alstonia scholaris on airway inflammation in rats. J Ethnopharmacol. 2016; 178:258-65. doi: 10.1016/j.jep.2015.12.022.

4. Hu TY, Ju JM, Mo LH, Ma L, Hu WH, You RR, et al. Anti-inflammation action of xanthones from Swertia chirayita by regulating COX-2/NF-κB/MAPKs/Akt signaling pathways in RAW 264.7 macrophage cells. Phytomedicine. 2019;55:214-221. doi: 10.1016/j.phymed.2018.08.001.

5. Shukla S, Mehta A, Mehta P,Vyas SP, Shivaprasad HN. In vivo immunomodulatory activities of the aqueous extract of bonduc nut Caesalpinia bonducella seeds. Pharm Biol. 2010;48(2):227-30. doi: 10.3109/13880200903085474.

6. Shukla S, Mehta A, Mehta P, Vyas SP, Shukla S, Bajpai VK. Studies on anti- inflammatory, antipyretic and analgesic properties of Caesalpinia bonducella F. seed oil in experimental animal models. Food Chem Toxicol. 2010;48(1):61-64. doi: 10.1016/j.fct.2009.09.015.

7. Gupta A, Khajuria A, Singh J, Bedi KL, Satti NK, Dutt P, et al. Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from Picrorhiza kurroa. Int Immunopharmacol. 2006;6(10):1543-9. doi: 10.1016/j.intimp.2006.05.002.

8. Kumar R, Gupta YK, Singh S, Raj A. Anti-inflammatory Effect of Picrorhiza kurroa in Experimental Models of Inflammation. Planta Med. 2016;82(16):1403-1409. doi: 10.1055/s-0042-106304.

9. Ram TS, Munikumar M, Raju VN, Devaraj P, Boiroju

NK, Hemalatha R, et al. In silico evaluation of the compounds of the ayurvedic drug, AYUSH-64, for the action against the SARS-CoV-2 main protease. J Ayurveda Integr Med 2021. https://doi.org/10.1016/j.jaim.2021.02.004.

10. Chopra A, Chavan-Gautam P, Tillu G, Saluja M, Borse S, Sarmukaddam S, et al. Coadministration of AYUSH 64 as an adjunct to Standard of Care in mild and moderate COVID-19: A randomised, controlled, multicentric clinical trial. Preprint available at: https://www.medrxiv.org/content/10.1101/2021.06.12.21258345v1

11. Reddy RG, Gosavi RV, Yadav B, Rai AK, Holay MP, Talekar M et al. AYUSH- 64 as an add-on to standard care in asymptomatic and mild cases of COVID- 19: A randomized controlled trial. Preprint available at: https://osf.io/pgraf/

12. Singh H, Srivastava S, Yadav B, Rai AK, Jameela S, Muralidharan S et al. AYUSH-64 as an adjunct to Standard Care in mild to moderate COVID-19: An open-label randomized controlled trial in Chandigarh, India. Preprint available at: https://osf.io/xyuas/

13. Bhardwaj P, Godatwar PK, Charan J, Sharma S, Shafi S, Chauhan N et al. Efficacy and Safety of Ayurveda Intervention (AYUSH 64) as add-on therapy for patients with COVID-19 infections – An open labelled, Parallel Group, Randomized controlled clinical trial. Preprint available at: https://www.medrxiv.org/content/10.1101/2021.08.10.21261836v1

14. Ministry of AYUSH, Government of India. Ayurveda Preventive Measures for self- care during COVID-19 Pandemic. Available from: https://www.ayush.gov.in/docs/Ayurveda%20Preventive%20Measures%20for%20self%20care%20during%20%20COVID-19%20Pandemic.pdf; 2021

15. Press Information Bureau, Government of India. Ayush Ministry launches nationwide distribution campaign of AYUSH 64 & Kabasura Kudineer. 7th May 2021. Available

from: https://pib.gov.in/Pressreleaseshare.aspx?PRID=1716729

16. Machhi J, Herskovitz J, Senan AM, et al. The Natural History, Pathobiology, and Clinical Manifestations of SARS-CoV-2 Infections. *J Neuroimmune Pharmacol*. 2020;15(3):359-386. doi:10.1007/s11481-020-09944-5

17. Carbone M, Lednicky J, Xiao SY, Venditti M, Bucci E. Coronavirus 2019 Infectious Disease Epidemic: Where We Are, What Can Be Done and Hope For. *J Thorac Oncol*. 2021;16(4):546-571. doi:10.1016/j.jtho.2020.12.014

18. Zacharioudakis IM, Prasad PJ, Zervou FN, et al. Association of SARS-CoV-2 Genomic Load with Outcomes in Patients with COVID-19. *Ann Am Thorac Soc*. 2021;18(5):900-903. doi:10.1513/AnnalsATS.202008-931RL

19. Jiang F, et al. Review of the clinical characteristics of coronavirus disease 2019 (COVID-19) *J. Gen. Intern. Med.* 2020;**35**:1545-1549. doi: 10.1007/s11606- 020-05762

20. Sharma R, Agarwal M, Gupta M, Somendra S, Saxena SK. Clinical Characteristics and Differential Clinical Diagnosis of Novel Coronavirus Disease 2019 (COVID-19). *Coronavirus Disease 2019 (COVID-19)*. 2020;55-70. Published 2020 Apr 30. doi:10.1007/978-981-15-4814-7_6

21. Romagnoli S, Peris A, De Gaudio AR, Geppetti P. SARS-CoV-2 and COVID- 19: From the Bench to the Bedside. *Physiol Rev*. 2020;100(4):1455-1466. doi:10.1152/physrev.00020.202

22. Ali N. Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. *J Med Virol*. 2020;92(11):2409-2411. doi:10.1002/jmv.26097

23. Wagner J, DuPont A, Larson S, Cash B, Farooq A. Absolute lymphocyte count is a prognostic marker in Covid-19: A retrospective cohort review. *Int J Lab Hematol*. 2020;42(6):761-765. doi:10.1111/ijlh.13288

24. Oliveira DS, Medeiros NI, Gomes JAS. Immune response in COVID-19: What do we currently know?. *MicrobPathog*. 2020; 148:104484. doi:10.1016/j.micpath.2020.104484

25. Yu C, Zhou M, Liu Y, et al. Characteristics of asymptomatic COVID-19 infectionand progression: A multicenter, retrospective study. *Virulence*. 2020;11(1):1006-1014. doi:10.1080/21505594.2020.1802194

26. Thakar A, Panara K, Patel F, Bhagiya S, Goyal M, Bhinde S, Chaudhari S, Chaturvedi S. Add-on Ayurveda Treatment for Early Stage COVID-19: A Single Center Retrospective Cohort Study From Gujarat, India. J Evid Based Integr Med. 2021;26:2515690X211020685. doi: 10.1177/2515690X211020685.

27. Cheng S.-C., Joosten L.A.B., Netea M.G. The interplay between central metabolism and innate immune responses. *Cytokine Growth Factor Rev.* 2014;25:707–713. doi: 10.1016/j.cytogfr.2014.06.008.

28. Hashimoto T, Perlot T, Rehman A, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature. 2012;487:477–81

29. Singh N, Suthar B, Mehta A, Nema N, Pandey A (2020) Corona Virus: An Immunological Perspective Review. Int J Immunol Immunother 7:050. doi.org/10.23937/2378-3672/1410050

30. Das, S. C., Bhadra, S., Roy, S., Saha, S. K., Islam, M. S., and Bachar, S. C. (2012). Analgesic and anti-inflammatory activities of ethanoic root extract of *Swertia chirata* (Gentianaceae). *Jordan J. Biol. Sci.* 5, 31-36.

31. Zhou HX, Li RF, Wang YF, Shen LH, Cai LH, Weng YC, Zhang HR, Chen XX,Wu X, Chen RF, Jiang HM, Wang C, Yang M, Lu J, Luo XD, Jiang Z, Yang ZF. Total alkaloids from Alstonia scholaris inhibit influenza a virus

replication and lung immunopathology by regulating the innate immune response. Phytomedicine. 2020;77:153272. doi: 10.1016/j.phymed.2020.153272.

32. Nguyen PQ, Ooi JS, Nguyen NT, et al. Antiviral Cystine Knot α-Amylase Inhibitors from Alstoniascholaris. J Biol Chem. 2015;290(52):31138- 31150. doi:10.1074/jbc.M115.654855

33. Najmi A, Javed D, Ray A, Sadasivam A. Review of Antiviral Activities Present in some Indian Medicinal Plants – Can they be used against SARS-CoV-2?. Int Arch BioMed Clin Res. 2020;6(4):RA1-RA5

34. Turaskar A, More S, Sheikh R, Gadhpayle J, Bhongade SL. Inhibitory potential of *Picrorhizakurroa* royle ex. benth extracts on phenylhydrazine induced reticulocytosis in rats. Asian J of Pharm Clin Res 2013;6(2):215-16.

35. Zhang DK, Yu JJ, Li YM, et al. A Picrorhiza kurroa derivative, picroliv, attenuates the development of dextran-sulfate-sodium-induced colitis in mice. *Mediators Inflamm*. 2012;2012:751629. doi:10.1155/2012/751629

36. Kumar V, Singh SB, Singh S. Pharmacological Perspectives of Ayurvedic herbs viz. Alstonia scholaris L., Picrorhiza kurroa, Swertia chirata and Caesalpinia crista against COVID- 19: A Mini-Review. Mini-Reviews in Organic Chemistry 2021;18:1-9. https://doi.org/10.2174/1570193X17999201102200944

37. Eric Wei Chiang entitled *Caesalpinia Crista:* A costal woody Climber with promising therapeutic values, Journal of Applied Pharmaceutical Science 8(3):133-140 10.7324/JAPS.2018.8319

38. Zhang S, Liu Y, Wang X, Yang L, Li H, Wang Y, et al. SARS-CoV-2 binds platelet ACE2 to enhance

thrombosis in COVID-19. J Hematol Oncol. 2020 Sep 4;13(1):120. doi: 10.1186/s13045-020-00954-7.

39. Ahirwal L, Singh S, Bharti V and Mehta A: Immunosuppressive effect *of Swertia chirata* Buch Ham. on Swiss albino mice. *Int J Pharm Sci Res* 2013; 4(12): 4763-68. doi: 10.13040/IJPSR. 0975- 8232.4(12).4763-68